

## **Supplemental Material**

### **Ambient PM<sub>2.5</sub>-Exposure Up-regulates the Expression of Co-Stimulatory Receptors on Circulating Monocytes in Diabetic Individuals**

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Individuals 2 and 7 had to be excluded from the analysis as one declined venipuncture and for one the withdrawn blood amount was not enough for flow-cytometry analysis.

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**Online Supplement Table 1. Cell surface marker function.**

<b>CD (cluster of differentiation) Marker</b>	<b>Major Cell Type Expressed</b>	<b>Receptor and Function</b>
CD23	Mature B cells; activated Macrophages; Eosinophils	Low affinity IgE receptor; mediates IgE allergic responses
FceR1	Mast cells; Basophils; Monocytes; Eosinophils	High affinity IgE receptor; mediates IgE allergic responses
CD80	APCs: Dendritic cells; Macrophages	Co-stimulatory receptor; antigen presentation to T cells
CD86	APCs: Dendritic cells; Macrophages	Co-stimulatory receptor; antigen presentation to T cells
CD40	APCs: Dendritic cells; B cells; Macrophages	Co-stimulatory receptor; activation of APCs; antibody production
HLA-DR	Macrophages; Dendritic cells; Monocytes	Major histocompatibility (MHC) class II receptor; antigen presentation
CD1a	APCs	Transmembrane glycoprotein receptor; presentation of lipid antigens to T cells
CD11b	Neutrophils; Macrophages; Monocytes	Complement receptor 3 for opsonised immune complexes; mediates complement mediated immune responses; phagocytosis; neutrophil migration
CD54/ICAM-1	Leukocytes; endothelial cells	Intercellular adhesion molecule; binds to leukocyte integrins/ligands and induces cell transmigration into tissue
mCD14	Monocytes; Macrophages	LPS receptor; mediates LPS-induced innate immune responses
CD16	Neutrophils; NK cells;	Fc gamma Receptor for IgG opsonized innate immune complexes; mediates innate immune responses
CD64	Macrophages; Monocytes	Fc gamma Receptor for IgG opsonised immune complexes; mediates innate immune responses; phagocytosis

APC = antigen presenting cells; ICAM-1 = intercellular adhesion molecule-1

**Online Supplement Table 2. Description of the study population clinical characteristics: current non-smoking subjects with type 2 diabetes mellitus.**

Clinical Characteristics	N=20 individuals
<i>Disease history</i>	Total number or mean
Type 2 diabetes mellitus	20
Time since diabetes diagnosis [yrs]	6.1
Hyperlipidemia	18
Hypertension	18
Past myocardial infarction	0
Coronary artery disease	4
Peripheral vascular disease	3
Cerebrovascular disease	1
Diabetic retinopathy	1
Diabetic nephropathy <sup>a</sup>	8
<i>Medication intake</i>	Total number
Sulfonylureas	9
Thiazolidinediones	6
Metformin	13
Statins	11
Aspirin	13
Beta-blockers	9
Angiotensin converting enzyme-inhibitors	11
Calcium-blockers	1
Diuretics	8
Angiotension II-receptor blocker	3
Estrogen	2

<sup>a</sup>Based on the screening urine (>30 µg albumin/mg creatinine) on spot collection.

**Online Supplement Table 3. Description of PM<sub>2.5</sub> and of meteorology parameters throughout the study period (19 Nov 2004 to 09 December 2005).**

Parameter	N	Mean	SD <sup>a</sup>	Min.	Max.
<b>Environmental Public Health Division Rooftop PM<sub>2.5</sub><sup>b</sup></b>					
PM <sub>2.5</sub> <sup>e</sup> [ $\mu\text{g}/\text{m}^3$ ], imputed	383	14.2	7.2	1.5	42.8
PM <sub>2.5</sub> <sup>e</sup> [ $\mu\text{g}/\text{m}^3$ ]	302	14.3	7.5	1.5	42.8
<b>Environmental Public Health Division Rooftop Meteorology</b>					
Air temperature [°C]	385	15.9	8.5	-6.5	31.5
Relative humidity [%]	385	62.5	16.6	25.1	97.7
Barometric pressure [hPa]	386	1001.2	6.5	981.4	1021.9

<sup>a</sup>SD: standard deviation

<sup>b</sup>PM<sub>2.5</sub>: particulate matter with a diameter <2.5 $\mu\text{m}$  (study period: 386 days)

**Online Supplement Table 4. Description of inflammation and cell surface markers (descriptive statistics were calculated from patient means).**

Parameter	N*	Mean	SD <sup>a</sup>	Min.	Max.
<i>Inflammation Parameters</i>					
Interleukin (IL)-6 [pg/ml]	79	3.5	2.2	1.3	9.0
Tumor necrosis factor (TNF) α [pg/ml]	79	1.7	0.9	0.8	4.7
<i>Monocytes (%)</i>					
CD23	75	6.0	6.6	0.7	27.5
FceR1	75	18.8	12.6	2.7	50.9
CD80	75	4.4	6.2	0.4	22.3
CD86	75	74.7	13.5	37.2	88.1
CD40	76	74.6	14.3	53.2	107.7
HLA-DR	75	78.1	14.1	50.2	92.9
CD1a	73	7.4	9.0	0.0	29.4
CD11b	76	87.7	4.7	77.5	93.4
CD54	75	69.4	17.7	30.6	91.3
CD14	76	73.5	9.1	59.6	87.8
CD16	75	25.3	6.2	12.6	37.4
CD64	75	76.1	9.1	61.3	89.6
<i>Neutrophils (%)</i>					
CD11b	76	95.5	5.8	76.1	99.5
CD14	76	23.2	11.9	3.1	53.7
CD16	78	102.0	25.3	90.4	209.1
CD64	77	11.3	13.6	0.7	55.9
<i>Monocytes (MFI)</i>					
CD23	76	3.3	2.1	0.9	8.8
FceR1	76	19.4	14.2	3.3	56.2
CD80	76	6.0	8.6	0.2	29.7
CD86	75	53.9	26.2	15.7	105.9
CD40	76	105.3	78.8	20.0	346.8
HLA-DR	76	121.4	57.4	32.8	215.2
CD1a	74	12.2	27.2	0.8	101.8
CD11b	76	131.4	85.6	50.4	417.8
CD54	76	19.7	9.0	9.0	40.1

CD14	76	557.3	208.7	246.2	1005.1
CD16	76	441.4	495.4	46.8	1810.0
CD64	76	73.6	38.7	17.0	196.2
<i>Neutrophils (MFI)</i>					
CD11b	76	101.0	65.9	45.7	340.4
CD14	76	10.1	6.1	3.4	28.5
CD16	76	4227.9	1660.7	2194.1	7774.63
CD64	76	3.7	3.7	0.3	17.2

\*20 patients with a maximum of 4 measurements each; <sup>a</sup>SD: standard deviation

**Online Supplement Table 5. Absolute changes of analyzed cell surface markers with 95%-confidence intervals based on a 1 $\mu\text{g}/\text{m}^3$  increase in PM<sub>2.5</sub>.**

	Lag 0	Lag 1	Lag 2	Lag 3	Lag 4
<b>Monocytes (%)</b>					
CD23	-0.21 [-0.49;0.06]	-0.11 [-0.41;0.20]	-0.22 [-0.59;0.16]	-0.19 [-0.61;0.23]	-0.05 [-0.40;0.30]
CD86	0.07 [-0.51;0.65]	0.39 [-0.21;1.00]	-0.08 [-0.85;0.69]	-1.04 [-1.83;-0.25]	0.07 [-0.61;0.75]
CD40	0.53 [-0.16;1.21]	0.35 [-0.36;1.05]	-0.36 [-1.25;0.54]	-0.32 [-1.27;0.63]	0.23 [-0.60;1.06]
HLA-DR	-0.14 [-0.74;0.46]	0.05 [-0.58;0.68]	-0.45 [-1.28;0.37]	-0.57 [-1.41;0.27]	0.38 [-0.36;1.11]
CD80	0.23 [-0.10;0.55]	0.27 [-0.08;0.63]	0.35 [-0.15;0.85]	0.27 [-0.29;0.84]	0.02 [-0.38;0.43]
CD1a	0.03 [-0.31;0.36]	0.02 [-0.37;0.40]	0.13 [-0.43;0.70]	0.05 [-0.51;0.61]	-0.07 [-0.47;0.33]
CD11b	-0.19 [-0.43;0.05]	-0.15 [-0.40;0.11]	-0.35 [-0.65;-0.04]	-0.31 [-0.64;0.03]	-0.14 [-0.45;0.18]
CD54	-0.55 [-1.26;0.16]	-0.19 [-0.97;0.59]	-1.06 [-2.04;-0.08]	-1.44 [-2.50;-0.37]	0.11 [-0.83;1.04]
FceR1	0.58 [0.14;1.01]	0.37 [-0.06;0.80]	-0.19 [-0.78;0.40]	-0.51 [-1.11;0.09]	-0.08 [-0.59;0.43]
CD14	0.22 [-0.19;0.62]	0.28 [-0.14;0.69]	-0.06 [-0.59;0.46]	-0.51 [-1.06;0.05]	-0.18 [-0.64;0.29]
CD16	0.14 [-0.15;0.42]	-0.06 [-0.36;0.24]	-0.31 [-0.69;0.08]	-0.26 [-0.67;0.14]	-0.04 [-0.39;0.30]
CD64	-0.23 [-0.65;0.20]	-0.11 [-0.55;0.34]	-0.47 [-1.01;0.06]	-0.33 [-0.88;0.23]	0.11 [-0.36;0.58]
<b>Monocytes (MFI)</b>					
CD23	-0.09 [-0.22;0.04]	-0.09 [-0.22;0.04]	-0.03 [-0.19;0.14]	-0.09 [-0.29;0.10]	0.18 [0.03;0.33]
CD86	-0.33 [-1.32;0.65]	0.01 [-0.93;0.95]	0.03 [-1.23;1.29]	-0.60 [-1.96;0.76]	1.14 [0.01;2.26]
CD40	1.23 [-1.71;4.16]	0.25 [2.67;3.17]	4.31 [0.56;8.06]	4.87 [1.00;8.75]	2.23 [-1.05;5.50]
HLA-DR	0.79 [-1.20;2.78]	0.18 [-1.78;2.15]	-1.48 [-4.01;1.05]	1.07 [-1.62;3.77]	2.23 [0.08;4.37]
CD80	0.37 [-0.15;0.88]	-0.16 [-0.74;0.42]	0.78 [0.19;1.37]	0.67 [0.06;1.28]	-0.29 [-0.84;0.25]
CD1a	0.59 [-0.04;1.22]	-0.25 [-0.77;0.27]	-0.07 [-0.56;0.42]	-0.19 [-0.61;0.23]	-0.07 [-0.41;0.26]
CD11b	-1.49 [-4.13;1.15]	-0.89 [-3.45;1.67]	-2.81 [-6.17;0.55]	-2.27 [-5.98;1.43]	0.43 [-2.47;3.32]
CD54	-0.02 [-0.33;0.28]	-0.21 [-0.53;0.12]	-0.42 [-0.86;0.01]	-0.31 [-0.77;0.16]	0.18 [-0.21;0.56]
FceR1	0.40 [-0.18;0.97]	-0.11 [-0.71;0.50]	-0.08 [-0.85;0.69]	-0.41 [-1.25;0.42]	-0.19 [-0.85;0.47]
CD14	-0.68 [-7.40;6.03]	0.52 [-6.47;7.52]	-3.76 [-13.59;6.07]	-2.61 [-12.16;6.94]	2.29 [-5.90;10.49]
CD16	-7.53 [-18.26;3.19]	-3.70 [-17.47;10.07]	-4.39 [-23.02;14.24]	-2.31 [-18.54;13.93]	-2.78 [-19.12;13.57]
CD64	0.04 [-1.00;1.08]	0.58 [-0.36;1.52]	0.06 [-1.23;1.35]	-0.70 [-2.04;0.64]	-0.54 [-1.66;0.58]

<b>Neutrophils (%)</b>					
CD14	0.25 [-0.24;0.73]	0.09 [-0.47;0.65]	-0.40 [-1.10;0.29]	-0.06 [-0.77;0.65]	-0.08 [-0.71;0.56]
CD16	0.22 [-1.26;1.69]	0.29 [-1.27;1.84]	1.30 [-0.66;3.27]	-0.30 [-2.41;1.81]	-0.25 [-1.97;1.46]
CD64	-0.57 [-1.02;-0.13]	-0.21 [-0.64;0.23]	-0.01 [-0.58;0.55]	0.17 [-0.42;0.75]	0.05 [-0.43;0.53]
CD11b	0.04 [-0.18;0.25]	0.05 [-0.18;0.27]	0.15 [-0.14;0.44]	-0.05 [-0.38;0.27]	-0.08 [-0.35;0.18]
<b>Neutrophils (MFI)</b>					
CD14	0.03 [-0.18;0.23]	-0.08 [-0.31;0.15]	-0.44 [-0.76;-0.11]	-0.17 [-0.45;0.11]	0.02 [-0.24;0.28]
CD16	28.73 [-30.39;87.85]	29.02 [-27.84;85.88]	-70.16 [-141.56;1.24]	-79.45 [-152.45;-6.46]	-9.85 [-81.89;62.19]
CD64	-0.02 [-0.16;0.12]	-0.02 [-0.14;0.10]	-0.03 [-0.22;0.15]	0.05 [-0.12;0.23]	0.09 [-0.05;0.24]
CD11b	-1.14 [-3.43;1.15]	-0.73 [-3.02;1.56]	-2.67 [-5.68;0.35]	-1.13 [-4.27;2.02]	2.01 [-0.52;4.53]

**Online Supplement Table 6. Pearson correlation coefficients between cell surface markers on monocytes (%-data) and inflammatory markers IL-6 and TNF $\alpha$ .**

Cell surface marker	CD23	FceR1	CD64	CD16	CD54	CD1a	CD11b	CD14	CD80	CD40	HLA-DR	CD86	IL-6	TNF $\alpha$
<b>CD23</b>	1	0.18	0.05	0.06	0.25	0.15	-0.13	-0.03	0.40	0.04	0.04	0.06	0.02	0.02
<b>FceR1</b>		1	0.11	0.15	0.10	0.74	-0.14	0.22	0.15	0.28	0.12	0.14	-0.13	-0.22
<b>CD64</b>			1	-0.18	0.67	-0.04	0.75	0.70	0.00	0.44	0.38	0.49	-0.13	-0.06
<b>CD16</b>				1	0.11	0.29	-0.14	0.01	0.02	0.28	0.00	0.22	0.16	-0.07
<b>CD54</b>					1	-0.07	0.54	0.37	0.01	0.32	0.50	0.55	-0.01	0.03
<b>CD1a</b>						1	-0.17	0.20	0.20	0.34	0.05	0.16	0.15	-0.12
<b>CD11b</b>							1	0.65	-0.08	0.28	0.23	0.40	-0.18	-0.01
<b>CD14</b>								1	-0.08	0.45	0.22	0.54	-0.23	-0.03
<b>CD80</b>									1	0.12	-0.26	-0.24	-0.02	0.00
<b>CD40</b>										1	0.34	0.56	-0.06	-0.13
<b>HLA-DR</b>											1	0.61	0.15	0.04
<b>CD86</b>												1	-0.11	0.02
<b>IL-6</b>													1	-0.03
<b>TNF<math>\alpha</math></b>														1

**Online Supplement Table 7. Pearson correlation coefficients between cell surface markers on monocytes (MFI-data) and inflammatory markers IL-6 and TNF $\alpha$ .**

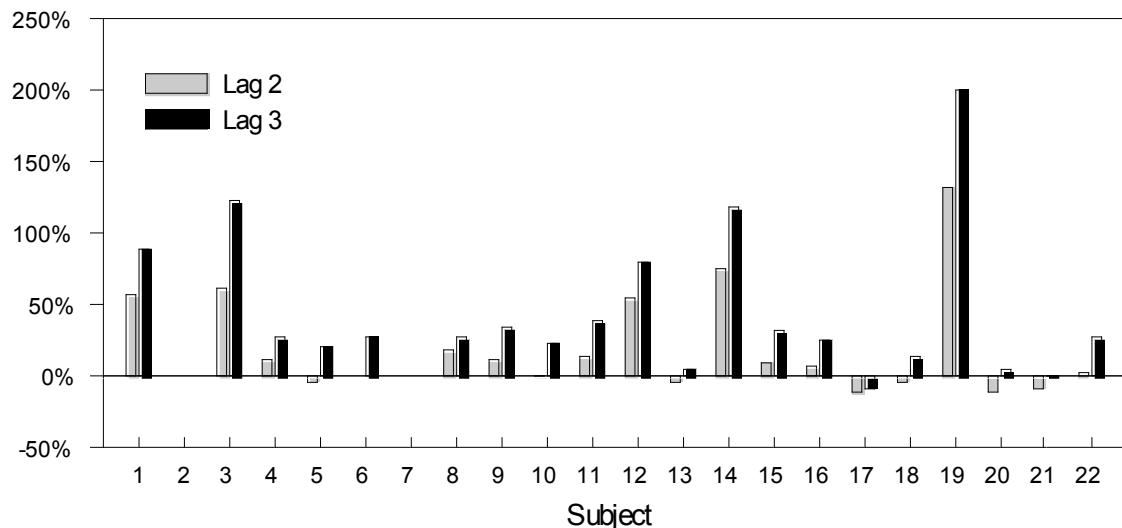
Cell surface marker	CD23	FceR1	CD64	CD16	CD54	CD1a	CD80	CD40	HLA-DR	CD86	CD11b	CD14	IL-6	TNF $\alpha$
<b>CD23</b>	1	0.54	0.22	-0.05	0.29	0.90	-0.05	0.11	0.25	0.26	0.17	0.06	0.23	-0.06
<b>FceR1</b>		1	0.21	-0.08	0.24	0.83	0.07	0.15	0.33	0.44	0.26	0.36	0.18	-0.10
<b>CD64</b>			1	-0.02	0.58	0.14	-0.14	0.53	0.57	0.40	0.35	0.39	0.05	0.09
<b>CD16</b>				1	-0.10	-0.04	-0.11	0.11	-0.26	-0.23	-0.01	0.08	-0.23	-0.11
<b>CD54</b>					1	0.43	-0.15	0.29	0.33	0.41	0.33	0.12	0.07	-0.02
<b>CD1a</b>						1	0.25	0.19	0.29	0.35	0.14	0.05	<b>0.56</b>	-0.08
<b>CD80</b>							1	0.49	0.04	-0.06	-0.18	-0.42	<b>0.41</b>	-0.08
<b>CD40</b>								1	0.33	0.43	0.06	0.08	0.04	0.01
<b>HLA-DR</b>									1	0.45	0.15	0.37	0.23	-0.13
<b>CD86</b>										1	0.47	0.55	0.14	0.03
<b>CD11b</b>											1	0.48	0.11	-0.03
<b>CD14</b>												1	-0.16	-0.09
<b>IL-6</b>													1	-0.03
<b>TNF<math>\alpha</math></b>														1

**Online Supplement Table 8. Pearson correlation coefficients between cell surface markers on neutrophils (%-data) and inflammatory markers IL-6 and TNF $\alpha$ .**

Cell surface marker	CD64	CD16	CD11b	CD14	IL-6	TNF $\alpha$
<b>CD64</b>	1	-0.14	-0.04	-0.16	0.05	0.11
<b>CD16</b>		1	0.32	0.03	0.13	-0.02
<b>CD11b</b>			1	-0.14	-0.07	0.05
<b>CD14</b>				1	-0.02	-0.12
<b>IL-6</b>					1	-0.03
<b>TNF<math>\alpha</math></b>						1

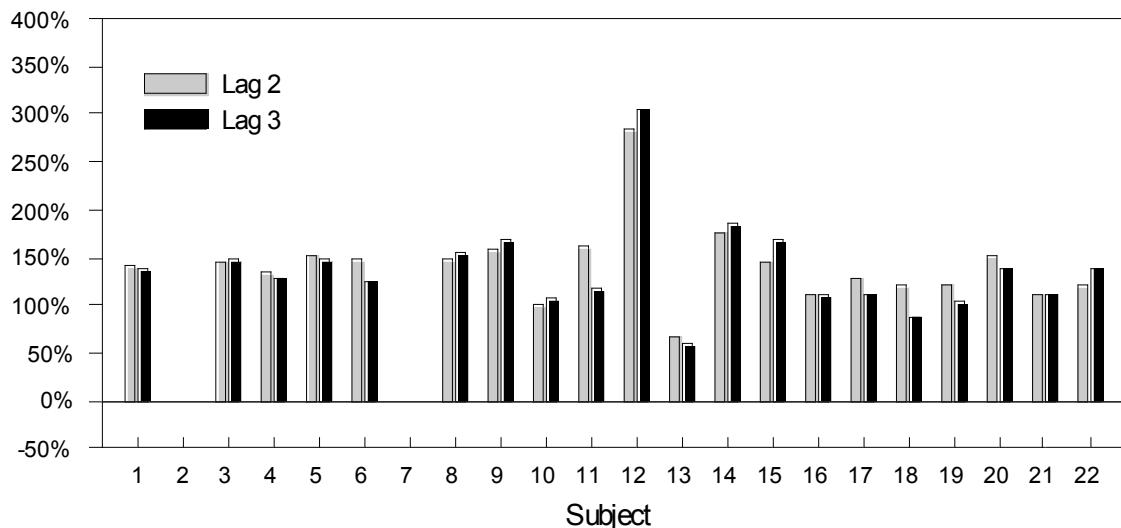
**Online Supplement Table 9. Pearson correlation coefficients between cell surface markers on neutrophils (MFI-data) and inflammatory markers IL-6 and TNF $\alpha$ .**

Cell surface marker	CD64	CD16	CD11b	CD14	IL-6	TNF $\alpha$
<b>CD64</b>	1	0.26	-0.05	0.12	-0.05	0.21
<b>CD16</b>		1	0.16	0.39	-0.10	-0.09
<b>CD11b</b>			1	0.30	0.13	-0.05
<b>CD14</b>				1	-0.02	-0.03
<b>IL-6</b>					1	-0.03
<b>TNF<math>\alpha</math></b>						1



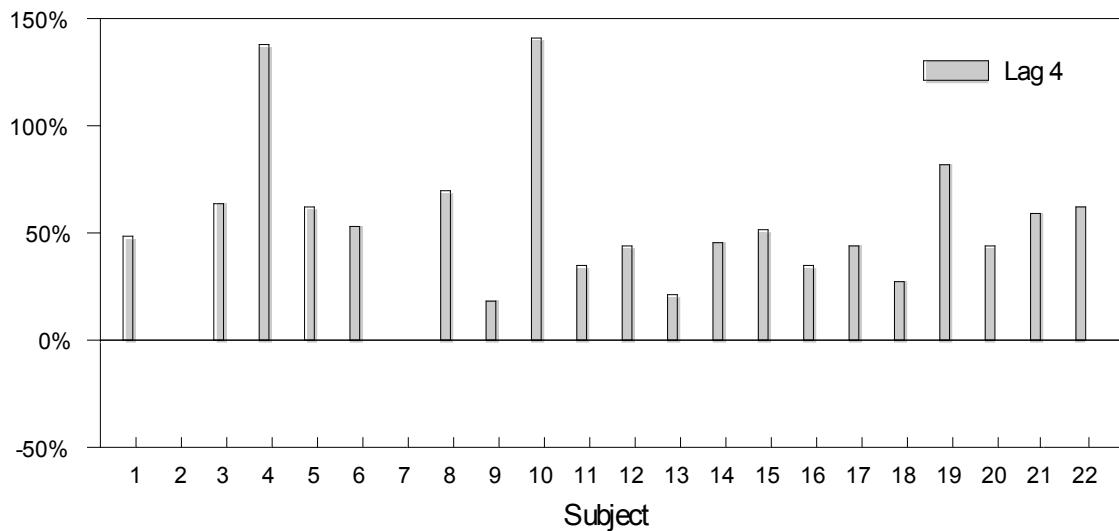
**Online Supplement Figure 1. Subject-specific associations (random slopes) with a  $10\mu\text{g}/\text{m}^3$  increment in  $\text{PM}_{2.5}$  (lag of 2 and 3 days) for CD40 monocytes (MFI).**

Subjects 2 and 7 had to be excluded from the analysis as one declined venipuncture and for one the withdrawn blood amount was not enough for flow-cytometry analysis.

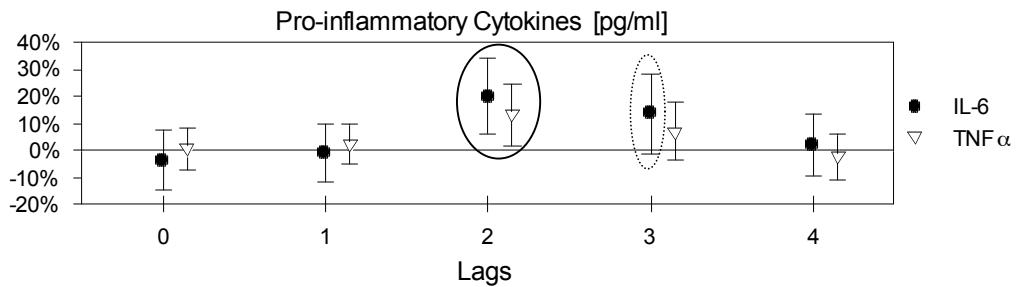


**Online Supplement Figure 2. Subject-specific associations (random slopes) with a  $10\mu\text{g}/\text{m}^3$  increment in  $\text{PM}_{2.5}$  (lag of 2 and 3 days) for CD80 monocytes (MFI).**

Individuals 2 and 7 had to be excluded from the analysis as one declined venipuncture and for one the withdrawn blood amount was not enough for flow-cytometry analysis.



**Online Supplement Figure 3. Subject-specific associations (random slopes) with a  $10\mu\text{g}/\text{m}^3$  increment in  $\text{PM}_{2.5}$  (lag of 4 days) for CD23 monocytes (MFI).** Individuals 2 and 7 had to be excluded from the analysis as one declined venipuncture and for one the withdrawn blood amount was not enough for flow-cytometry analysis.



**Online Supplement Figure 4. Effect estimates for IL-6 and TNF $\alpha$  with 95%-confidence intervals for immediate and delayed associations with PM<sub>2.5</sub> (Schneider et al. 2010).**

**Reference:**

Schneider A, Neas LM, Graff DW, Herbst MC, Cascio WE, Schmitt MT et al. 2010. Association of cardiac and vascular changes with ambient PM<sub>2.5</sub> in diabetic individuals. Part Fibre Toxicol 7:14.